Electronic supplementary material to:

"The influence of L-carnitine on the formation of preneoplastic and atherosclerotic lesions in the colon and aorta of male F344 rats"

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Assessment of L-carnitine stability

L-Carnitine (5 g/l, 31 mM) in a rat bottle was stored in an airflow cabinet under experimental conditions (see Materials and Methods "Animals, housing and diet") for up to 7 days. Three 1 ml samples were taken after 0, 3, 48, 72, 96, 120, 144 and 168 hours incubation time and stored at -20 °C until measurement. Quantification of L-carnitine in the samples was carried out by liquid chromatography (LC) and mass spectrometry (MS) detection after positive electrospray ionization. The used LC-10-ADvp LC system (Shimadzu, Langenfeld, Germany) consisted of one SCL-10Avp controller, 2 pumps (LC-10ADvp or LC-10AD), a column oven (CTO-10ASvp) set to 40 °C and a degasser (DGU-14A). Samples were kept at 4 °C in a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland) with an injection loop of 20 µl, the

injection volume being 10 µl. A RP18 Spherisorb S3 column (1 x 150 mm, 3 µm) (Waters, Eschborn, Germany) with an isocratic mobile phase consisting of acetonitrile/aqueous 0.1 % formic acid (v/v 5/95) at a flow rate of 100 µl/min (acetonitrile (HPLC grade) Carl Roth, Karlsruhe, Germany; formic acid (98-100 % purity) Applichem, Darmstadt, Germany) was used for LC separation. The analyses were performed using a Micromass LC-Quattro triple quadrupol mass spectrometer (Waters) in selected reaction monitoring mode. The transition m/z 162 → 85 was used for the quantification of L-carnitine. The instrument was operated with the following parameters: capillary voltage 3.5 kV, cone voltage 30 V, extractor voltage 4 V, RF lenses 0.2 V, source temperature 130 °C, desolvation temperature 400 °C, nebulizer gas flow 125 l/h, desolvation gas flow to 750 l/h, low and high mass resolution 1 and 2 12.5, collision energy 20 eV, ion energy 1 3.0, ion energy 2 0.5, entrance potential 2, exit potential 2, dwell time 50 ms and multiplier 500. Nitrogen was used as nebulizer and desolvatisation gas and argon as collision gas. Quantification was carried out by an external calibration with L-carnitine (≥ 98 % purity; Sigma-Aldrich, Schnelldorf, Germany) as standard. Instrument controlling and data analysis was performed with MassLynx 4.1 (Waters). Prior to injection the water samples were diluted 1:1000 with the mobile phase.

As is shown in Supplementary Table 1, L-carnitine is stable for 7 days under the given experimental conditions.

Supplementary Table 1 L-Carnitine concentrations in the drinking water after a 7-day incubation under experimental conditions

Incubation time [h]	L-Carnitine concentration [µM; mean ± SD; n = 3]
0	31.9 ± 1.8
3	34.8 ± 2.3
48	34.0 ± 2.5
72	34.5 ± 2.8
96	33.8 ± 0.9
120	32.4 ± 2.6
144	29.1 ± 1.4
168	31.5 ± 1.7

Specifications of the animal feed

Ingredient	Content [mg/kg, except where noted otherwise]
Crude ash	69364.89
Crude fat	40803.01
Crude fiber	60518.48
Crude protein	191970.4
Disaccharides	49463.05
Polysaccharides	358852.33
Moisture	112946.89
Metabolizable energy	3188.487 kcal/kg
Alanine	8557.75
Arginine	11503.05
Aspartic acid	15905.35
Cystine	3171.1
Glutamic acid	38495.6
Glycine	8345.1
Histidine	4465.1
Isoleucine	7560.45
Leucine	13416.5
Lysine	8026.06
Methionine	2738.23
Phenylalanine	8326.5
Proline	12427.3
Serine	9127.55
Threonine	6611.3
Tryptophan	2458.45

Ingredient	Content [mg/kg, except where noted otherwise]
Tyrosine	5962.05
Valine	8858.1
Biotin	0.25
Choline chloride	699
Folic acid	2.335
Nicotinic acid	36
Pantothenic acid	21
Vitamin A	15000 IU/kg
Vitamin B1	18
Vitamin B12	0.024
Vitamin B2	12
Vitamin B6	9
Vitamin C	36
Vitamin D3	600 IU/kg
Vitamin E	110.35
Vitamin K3 as menadione	3
Aluminium	97.963
Calcium	7114.94
Chlorine	3541
Cobalt	0.351
Copper	13.582
Fluorine	2.192
Iodine	1.623
Iron	198.037
Magnesium	2436.93

Ingredient	Content [mg/kg, except where noted otherwise]
Manganese	97.686
Molybdenum	1.129
Phosphorus	5090.56
Digestible phosporus	1537.5
Potassium	9214.9
Selenium	0.265
Sodium	2156.565
Sulfur	1198.2
Zinc	94.876
Palmitic acid C-16:0	3581.475
Stearic acid C-18:0	1094.3
Oleic acid C-18:1	6292.225
Linoleic acid C-18:2	14896.725
Linolenic acid C-18:3	2038.7
Arachidic acid C-20:0	40
Eicosaenic acid C-20:1	50